

PROJECT NUMBER: 6908  
PROJECT TITLE: Smoke Condensate Studies  
PROJECT LEADER: A. H. Warfield  
PERIOD COVERED: April, 1989

## I. TSNA PRECURSORS

- A. Objective: To determine the precursors of MS TSNA.
- B. Results: Data collection was continued as part of an aging experiment on water-washed burley filler. Time-course filler TSNA and alkaloid data are now available after storage at -30° and 45°C for 21, 42 and 63 days. Thus far there has been little change in endogenous NNN and NAT, but there is a definite trend towards higher NNK in the 45°C sample with time. There has been no change in alkaloid levels (measured by KOH/methanol extraction followed by GC/NPD) found in the filler over the observation period. Only one MS TSNA determination has been made thus far (42 days), and these data show no change in MS NNN or NAT, but there is an apparent increase in MS NNK. More data will have to be collected to determine whether this increase is significant.

Additional evaluations of stored RLs from the Crossed Solubles-Base Web (CSBW) study were carried out. Filler NNN and NAT levels of BuCEL on BuBW increased by approximately 40% after one year at room temperature, while filler NNK increased by 50%. MS NNN and NAT were only slightly higher after the aging period relative to the time immediately after preparation of the RL, but MS NNK was increased by 87%. Similar studies were carried out on the BuBW used to prepare the RL, but the values obtained were much lower than those obtained when the RL was analyzed, and no definite conclusions could be drawn.

- C. Plans: The aging study samples will be analyzed for filler TSNA every three weeks and MS TSNA every six weeks until July, 1989.

D. Reference:

Haut, S. A. Notebook No. 8768, p. 108.

## II. ORIENTAL TOBACCO STUDIES

- A. Objective: To determine if the inhibition of TSNA formation/pyro-synthesis observed for oriental tobacco is due to agronomic effects.
- B. Results: Nicotine and minor alkaloid data on eight oriental varieties were obtained by capillary GC/NPD. The analyses were performed by Bob Levins (Project 6912). Total alkaloid data were obtained from ARD on the same samples. There was considerable variation in individual alkaloid levels among the different varieties. Nicotine levels ranged from 0.3% to 2.9%, while normicotine levels ranged from 0.005% to 0.07%.

C. Plans: Filler and MS TSNA data as well as further ARD analyses will be completed on the eight oriental varieties, and the individual varieties will be evaluated with respect to their likely level of inhibition of MS TSNA.

D. Reference:

Keene, C. K. Notebook No. 8754, p. 140.

### III. ORIENTAL INHIBITOR STUDIES (CEL)

A. Objective: To determine the causative agent(s) responsible for the reduction in MS TSNA observed when oriental (Or) CEL is added to burley (Bu) CEL and applied to Bu base web (BW), relative to a control RL prepared from BuCEL on BuBW.

B. Results: Studies were continued in which the effect of OrCEL and sugars (present in special RLs) on sidestream (SS) as well as MS TSNA were determined. The test RLs were made from mixed Bu and Or CELs and BuCEL + "sugars" on BuBW. Corresponding controls were made from two levels of BuCEL on BuBW. ("Sugars" consisted essentially of the calculated amounts of individual sugars present in the OrCEL.) Initial results were reported in February. The inhibitory effect of OrCEL on SS TSNA appeared to be much smaller than that on MS TSNA. In addition, an increase in MS TSNA deliveries was noted on aging of the RL containing OrCEL.

Current results show that a two-fold increase in MS TSNA from BuCEL on BuBW occurred during a one year of storage at room temperature after the RL was prepared. Addition of OrCEL caused a lower initial MS TSNA level and greatly attenuated the increase in MS TSNA on aging. Addition of sugars also lowered initial MS NNN and NAT, but (in contrast to OrCEL) resulted in a further decrease in MS NNN and NAT on aging, while there was a slight concomitant increase in MS NNK. The observation (previously reported) that OrCEL caused reduction of MS NNK, whereas sugars had no effect on initial MS NNK, was taken as an indication that OrCEL may contain inhibitors capable of reducing MS NNK. These inhibitors are apparently different from those derived from the sugars contained in OrCEL. There was an indication that the total increase in MS NNK that occurred in the one year period (in the absence of an added inhibitor) occurred in a 1.5 month period, whereas the increase in MS NNN and NAT appeared to be linear with time. Insufficient data are presently available to confirm the latter observations.

SS TSNA data on the above samples are now available after an elapsed time of 1.5 and 7 months since the RLs were prepared. As reported earlier there was an increase in SS TSNA with time, but the slope of the increase was much smaller than that observed for MS TSNA.

Because of the fact that sugars added to BuCEL (which contains high levels of soluble ammonia) have been observed to effect

reductions in MS TSNA, it has been postulated that sugar-ammonia reaction products may be responsible for the reductions. Bright filler contains high levels of reducing sugars but little soluble ammonia. Therefore, ammonia as diammonium phosphate (DAP) was added to bright CEL at a target level of 0.37% ammonia in the RL. (BuCEL + "sugars" on BuBW contained 0.53% ammonia). The control was a bright RL containing 0.06% ammonia. The levels of individual sugars were similar to those in the BuCEL + "sugars" RL except that there was more glucose in the latter. However, addition of ammonia at this level did not affect the levels of either MS or SS TSNA obtained on smoking cigarettes made from these RLs.

- C. Plans: Data collection on the BuCEL/OrCEL/"sugars" on BuBW aging study will continue. An RL has been prepared from mixed Br and Bu CELs on BuBW in order to further investigate the possible contribution of ammonia-sugar reactions or related reactions to the inhibition of MS TSNA observed on addition of OrCEL to the BuCEL on BuBW RL.

D. Reference:

Morgan, W. R. Notebook No. 8579, pp. 158-163.

IV. ORIENTAL INHIBITOR STUDIES (ORGANIC EXTRACTS)

- A. Objective: To determine whether the causative agent(s) responsible for the reduced levels of MS TSNA observed for Or tobacco can be removed with organic solvents and applied to other fillers as a means of decreasing the MS TSNA levels delivered by these fillers.

- B. Results: With the assistance of R. Izac, a blended sample of oriental tobacco was extracted sequentially with methylene chloride (MeCl) and methanol (MeOH). These extracts were applied to two test fillers: burley filler extracted with 5% EtOH/hexane (ExBu), and BuBW. The extracted filler is low in nicotine and endogenous TSNA but still contains minor alkaloids, salts, and other water solubles. BuBW is devoid of all the above but still produces pyrosynthetic NNK. Data obtained thus far have resulted in reductions of 34, 30, and 11% in MS NNN, NAT, and NNK, respectively, for ExBu filler treated with the MeCl extract.

- C. Plans: The remaining samples will be evaluated for MS TSNA deliveries.

D. Reference:

Haut, S. A. Notebook No. 8768, p. 108.

V. MISCELLANEOUS AND SUPPORT STUDIES:

- A. Objective: To conduct studies of the TSNA content of filler and/or MS smoke as necessary to support other PM programs.

B. Results: MS TSNA data were obtained on machine-made cigarettes consisting of RCB and full-flavored blend. The RCB included washed burley stems (control) or washed burley stems combined with Post-ART bright stems. The data have been discussed in a memo.

C. Reference:

Haut, S. A. Notebook No. 8768, p. 108.